Different Chemical Compound Profiles of Indonesian Coffee Beans as Studied Chromatography/Mass Spectrometry

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Abstract. Coffee is a favourite beverage worldwide, with all of its benefits. The taste and flavour of coffee depending on the chemical components of the coffee. And the presence of chemicals in the coffee ground depends on the original tree as well as on processing. In this report, some volatiles from ground and brewed coffee beans of Robusta, Arabica, and peaberry coffee beans were extracted to be described after chemical analysis. Mainly gas chromatography/mass spectrometry methods were the main spectroscopy utilized to obtain chemistry information that was taken from hot brewed coffees. The result showed that peaberry coffee beans were very rich in components compared to others. Arabica coffee beans also had stronger in taste and flavour compared to robusta as seen from the profile compositions of chloroform and *n*-hexane extracts. Better processing would also be suggested for each type of coffee beans.

Keywords: Robusta coffee, arabica coffee, peaberry coffee, extraction, component profile, GC/MS.

1. Introduction

Drinking coffee is a worldwide culture, and the flavour might be the centre of interest [1,2]. Discussion about coffee gives impacts to quality of social life due to caffeine content that stimulates the human body to work for longer. The changes in biochemistry in a human body is something else to discuss while chemical content of coffee, besides caffeine is always interesting to analyze. They contribute to the taste and flavour of coffee during brewing. Possible changes during coffee beans processing increase the creativity of a barista in seeking the best coffee for each customer. Cold brewed coffee is now becoming a lifestyle too, and some people like this type of coffee better. The taste and flavour of coffee were acknowledged as extremely complicated to analyze [3], arises from many compounds are inherently present in the plant. Most of the changes in chemical compounds of

coffee occur during roasting [1,4]. However, there is so much complexity in the fine analysis of coffee flavours due to the rich chemical content in coffee beans. The types of coffee, as well as the area of origin, the altitudes of the field and type of lands, do give impact to the quality, apart from the process of making coffee itself [5]. The origin of coffee beans and the genetic type as well as region give impact to the chemical components of the coffee [6]. On the other hand, the analysis of coffee flavour still gains much interest from culinary people due to the possibility to play with different components leading to the uniqueness of taste. Therefore chemistry analysis was done and validated to describe the differences between coffees from different types and treatments [7–9]. More emphasis on extraction methods of coffee brewing was also reported [10,11]. More analysis of such method will give statistic as a powerful tool to analyze complicated information [7,8]. A lot of analytical chemists bring chemometry to the analytical data to extract the different sets of data from coffee analysis, especially using infrared and near-infrared method [7,12].

Besides infrared and UV-visible spectrometry, coffee and other natural secondary metabolites, chromatography is also utilized [8,10,13,14]. Separation of components extracted allowed better characterization of each type of coffee. Chromatography analysis after extraction treatments was once well described [15]. The utilization of stationary and carrier gas or liquid was also a good theme for the analytical chemist. Calculation of components is also enabled in this method, to see possible treatment to be applied for special purposes with the coffee during brewing. Statistical methods were also applied together with chromatography in some analysis. Statistics using chemistry data, the chemometry, is an emerging method in modern analysis, followed by metabolomics. The tendency of modern analysis is the work without the complexity of separation processes. The result would be far from structural and chemistry description, but more complex with material mixtures, which is closer to the original states in nature. In this case, the complicated data must be treated differently. This approach is suitable to describe the chemistry of coffee to society.

Indonesia, which is located in the tropical area, is preferable to cultivate coffee plants, as written in Wikipedia and social media. There are also two types of coffee beans usually planted in Indonesia, the Robusta and Arabica types [16–18]. The Robusta coffee (*Coffea canephora*) is known as coffee with low acidity and high bitterness, and Arabica coffee (*Coffea arabica*), which is grown more in the world, has more acid content in its beans. Robusta coffee is the more common raw material for instant coffee. The two types of roasted coffee will be analyzed in these experiments, together with "*kopi lanang*" which literally means "male coffee" or pea-berry coffee beans, that becomes popular in this country. This pea-berry bean is the single bean found in a coffee tree, the number is not many since this is the abnormal beans exist in one tree. That is why the price is usually higher.

The aim of the study is to describe the coffee chemical components after roasting. The chemical contents for three of them would be extracted by hot water as it is brewed, and then extracted by different solvents (*n-hexane* and *chloroform*), to take the non-polar and polar components. Some components would be extracted in both but their profiles are diverse, after being separated by chromatography method followed by mass spectrometry determination.

2. Methods

The roasted coffee beans were bought from the local roastery (Mreneo Roastery) in Malang region, Indonesia. However, the samples of coffee beans were taken from the original lands and areas around Indonesia. The roasting process follows the common roasting method. The chemicals used for all experiments are from E. Merck and Sigma-Aldrich, p.a. grade. Chromatography and mass spectrometry used was QP2010Plus from Shimadzu and IRPrestige-21, also from Shimadzu.

2.1. The preparation of the extraction process

There were some steps of extraction done to obtain all information. The first extraction was done using 50 mL of boiled water to 2 grams of ground coffee beans, stirred and let settled around 30 minutes. After filtration, half was given *n*-hexane and the other Chloroform of the same quantity prior to parting by separating funnels. The organic extraction was done since water cannot be used in

chromatographic separation using normal semi-polar column. The extracts were filtered again and put into small vials before column injection to the instrument for gas chromatography. Some dilution was done when necessary.

2.2. The characterization of extract using GC/MS

Chromatography experiments were set up with this setting: oven temperature 70 ºC for 5 minutes then increased to 300 ºC with 5-degree pro minute rate, then holding it until 19 minutes. Injection temperature was 300 ºC, ion source temperature was 250 ºC, interface temperature 305 ºC. The flow rate was 25.5 cm^3/sec. The components were analyzed and matching to Wiley 8 library. Components with minimum 80 of similarity index (SI) were considered in this report.

3. Results and Discussion

The result of extraction by hexane and chloroform showed the ability of both solvents to take all components of coffee beans out of the tissue. Hexane extraction has the aim to take all nonpolar components after hot brewing process, while the polar constituents were taken by chloroform (Figure 1a). Actually, some of the components were taken by both solvents, and they were divided according to their distribution constant under n-hexane and water as well as water and chloroform [19]. This constant depends on many other parameters, including pH and temperatures. That is why both of the extracts contained some identical components, but in different compositions in the profiles.

However, some components could not be taken by polar or nonpolar solvents when they are very polar or ionized in water. Hot water helped the extraction by opening some porous pockets in the interior beans and release some dissolved constituents, such as caffeine and tannins as well as terpenoids [20,21]. Some of them are bigger components and cannot be vaporized in the GC systems as they are not volatile enough. Other big constituents such as carbohydrates and proteins would be filtered as they were gone as aggregates after extraction (Figure 1b) prior to separation using separating funnel.

(a) (b)

Figure 1. Extraction by *n*-hexane and chloroform from hot brewed ground coffee (a) *n*-hexane layer is the upper layer (a, left) and chloroform layer is the lower layer (a, right), aggregation of organic bigger molecules, filtered from the extract (b) before separation using separating funnel.

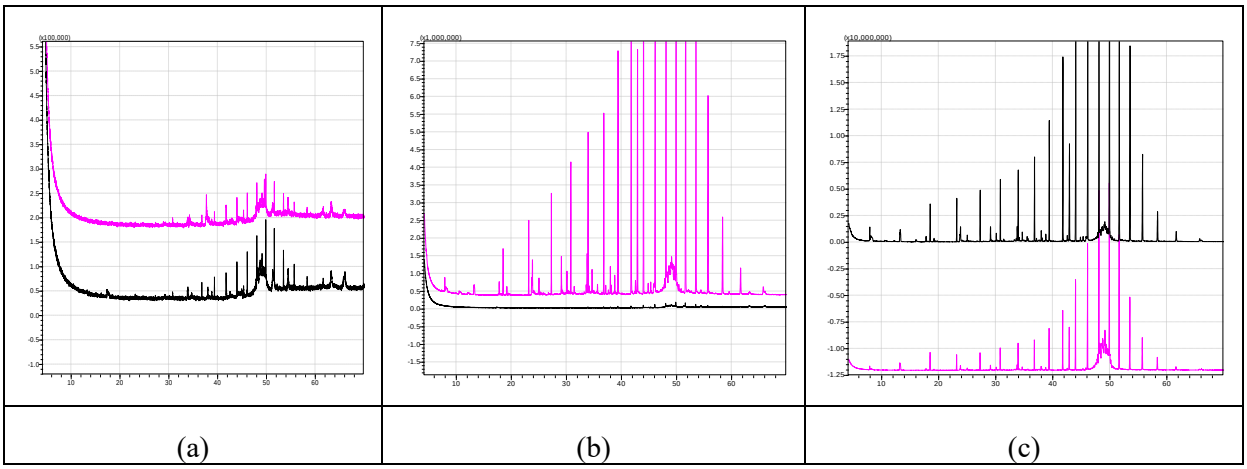
The choice of *n*-hexane and chloroform was based on its polarity so that most of the components can be taken from the hot water solution. The use of two types of solvents was already used several times to take polar and non-polar components of plants before [22,23]. However, the proportion of each component in water and *n*-hexane or chloroform vary from compound to compound [19]. Also, it is possible that the same component was extracted by *n*-hexane as well as by chloroform, although the composition must be different. Components with higher molecular weight would also be extracted. However, after settling down, they are moving towards granulation while they were not very soluble anymore. The gel-form granules appeared and separated easily (Figure 1b). It might consist of

carbohydrates and proteins which are partly water soluble. The rest would be secondary metabolites which are responsible for the flavour, taste, as well as aroma of the coffee. The richness of coffee depends on these natural chemicals.

Apart from proteins and most carbohydrates, caffeine, reducing sugars, chlorogenic acids and other bigger chemicals stuff in the coffee beans, there are a lot more chemicals involved in the taste and flavour of coffee. Some can be extracted well to analyze but it is impossible to get all chemicals out of the coffee beans matrix. Having different types of solvents for complete extraction was in fact presented most of the chemical compounds out of the matrix. This was aimed to analyze the contribution of each compound to the taste and flavour of the coffee.

From the chromatogram above we can see that the power of chloroform to extract components in coffee beans compared to n-hexane. This also indicates the components extracted are mainly polar compounds. Only peaberry coffee has very rich components, for both polar and non-polar compounds, compared to Arabica and robusta coffee. Not many components present in the robusta extract for both solvents, and this would produce mild taste and aroma for this coffee. Arabica coffee beans yield more components from chloroform extraction indicating more polar components present in this type of coffee beans. More polar components can be alcohol or phenolic compounds which have more power in antioxidant properties as well as other good properties from coffee drinks. But apart from those two coffee bean, peaberry coffee consist of more types of components, both polar and non-polar, as can be seen in Figure 2c. Those which can be taken from n-hexane solvents can contribute to the "oily" taste of brewed coffee. Of course, there must also be possibilities that the same components can be extracted by both chloroform and n-hexane solvents.

Arabica coffee beans showed different profile compared to robusta coffee beans as seen from the chromatogram obtained (Figure 2). Not many chemical compounds could be taken from robusta coffee beans, using both *n*-hexane and chloroform (Figure 2a). It means the number of chemicals in hotly brewed robusta coffee beans were not that much compared to Arabica or peaberry beans. However, the other type of components like carbohydrates, proteins, bigger components, have not been investigated.



(a) (b) (c)

Figure 2. Chromatograms of chloroform (pink) and *n*-hexane extracts (black) of robusta coffee (a), Arabica coffee (b) and peaberry coffee (male coffee) (c).

Chloroform extract of Arabica coffee beans was richer compared to hexane extract (Figure 2b). More polar components present contributes to the flavour and aroma of Arabica coffee brewed. There are some carboxylic acids and also some long chained esters present according to Wiley library of the mass spectrometry results (Table 2 and 3). The acids contribute to the acidity of the Arabica coffee, and the esters contribute to giving good smells of roasted coffee beans. Most of the components can also present in hexane extract due to a long chain or branched chain. In peaberry coffee, most of the

components could be taken by both polar and non-polar solvents. Some acids, esters, as well as phenolic compounds, present in both extracts, and this might be the goodness of peaberry coffee beans and make the price of this type a lot higher compared to others. Due to the strong flavour, this type of coffee is called by masculine stylishness in Indonesia. The comparison of chemical profiles of the three coffee types can be seen clearly in Figure 2.

There are also components which are unresolved. The very broad peaks around 50 minutes of retention time in all chromatograms indicate the complexity in separating the components. The components themselves must be very similar so that they are not easily separated. In the next experiments, these groups of components would be elaborated, since the inherent properties of coffee might rely on this.

As automatically each component separated in the chromatography column will undergo fragmentation in the ionization chamber in the mass spectrometer, the pattern of fragmentation was recorded and matched with Wiley 8 library. Some could be matched with very high similarity, but some would be poor. In the poorly matched mass spectrum, the component might be not well resolved so that the mass spectra were not from a pure component. According to Willey library, the composition of both solvents main compounds for three of the coffee beans are presented in Table 1 below, only the well-matched spectrum were chosen.

Table 1. The main components and their amount in mixture of Robusta, Arabica and Peaberry coffe in chloroform extract

robusta	arabica	peaberry coffebeans
heptadecane, 2,6,10,15-tetramethyl-	1,1,1,3,5,7,9,11,11,11-decamethyl-5-[(trimethylsilyl)oxy]hexasiloxane #	cyclopentasiloxane
1,2-benzenedicarboxylic acid, dioctyl ester	heptadecane	1,1,1,3,5,7,9,11,11,11-decamethyl-5-(trimethylsiloxy) hexasiloxane
2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-	3,4-dihydroxymandelic acid-tetratms	hexanedioic acid
2,2,2-trichloroethyl 3-oxobutanoate	nonadecane	1-ethoxy-3,3,3-trimethyl-1-[(trimethylsilyl)oxy] disiloxanyl tris (trimethylsilyl) orthosilicate
1h-purin-6-amine, [(2-fluorophenyl)methyl]-	2-[(2,4,4,6,6,8,8-heptamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocan-2-yl)oxy]-2,4,4,6,6,8,8,10,10-nonamethyl-1,3,5,7,9,2,4,6,8,	dioctyl ester
2(5h)-furanone, 5-ethyl-	1-isopropoxy-3,3,3-trimethyl-1-[(trimethylsilyl)oxy]disiloxanyl tris(trimethylsilyl) orthosilicate # hexanedioic acid, dioctyl ester 1,2-benzene dicarboxylic acid, diisononyl ester	1,2-benzenedicarboxylic acid

On the other hand, carbohydrates and proteins, and other essential content of coffee like caffeine and chlorogenic acids cannot be analyzed by this method. The bigger components will be analyzed

elsewhere. Protein and carbohydrate need liquid chromatography since they cannot be made gas during separation. Only aroma and taste by smaller molecules can be listed. Moreover, in the case of incomplete separation, the mass spectra would not match with mostly compounds in the library so that the similarity index were low and the data were also excluded.

Table 2. Name of components and their amount in the mixture of Robusta, Arabica and Peaberry coffee in n-hexane extract

Robusta hexane	Arabica hexane	Peaberry hexane
1,2-benzene dicarboxylic acid, dinonyl ester	pentadecane, 2,6,10,14-tetramethyl-	cyclopentasiloxane, decamethyl-
dodecane, 2,6,11-trimethyl-	nonane, 5-(2-methyl propyl)-	hexanedioic acid, dioctyl ester
iron, tricarbonyl[(o,1,2,3-.eta.)-methyl 2-propenoate]-	octadecane	1,2-benzene dicarboxylic acid, dinonyl ester
2-propenoic acid, butyl ester	1,2-benzene dicarboxylic acid, bis(2-methyl propyl) ester	heptasiloxane, hexadecamethyl-
undecane, 4,8-dimethyl-	1,2-benzene dicarboxylic acid, dinonyl ester	1,2-benzene dicarboxylic acid, dinonyl ester
3-heptanone, 4-methyl-	1h-purin-6-amine, [(2-fluorophenyl)methyl]-	cyclododecasiloxane, tetracosamethyl-
1,2-benzene dicarboxylic acid, dinonyl ester	butane, 1,1'-oxybis-	
2-pentanone, 5-hydroxy-		
1-(1,4,5,6-tetrahydro-2-pyridinyl)ethanone #		
allyl tetrasulfide		

The extraction process too actually was a single extraction method. There were many components remained in the water part solvent according to the distribution coefficients [19]. Using repeated extraction or continuous extraction like some extraction using soxhlet apparatus will yield more compounds into the extract. That is why coffee machines will give delicious coffee when used to make many cups of coffee rather than a single cup only. Most of coffee brewing methods rely on many times of extractions or continuous extraction using hot water.

The result of coffee chemical compounds is always interesting. Modern food science enables one to manipulate the flavour, even from the beginning, for example for making decaffeinated coffee from the plant [24]. Even though it was already reported a long time before, the effort to change chemical contents from the root of their biosynthesis, which is called genetic engineering. The real extract can also be made perfumes or odour source for daily use. Some aroma from a coffee can be used as perfumes or room odour or insect repellant too [25–27]. The richness of coffee beans will go further in the future, not only for favourite industrial drinks.

However, there must be critical analysis as well from the methodology. There is no best method without weakness. Extraction cannot fully take all of the chemicals out of the matrix. While the matrix itself is also chemicals. Cellulose and lignin are also retaining chemicals by physical attractions. The surface tension of both solvent as extractor must be able to dissolve chemicals from the place it contained in the matrix. That is why coffee beans should be ground into powder to make possible for the solvent molecules to come into a deeper area. Next, not all the compounds can be made vapour and delivered in the column, some of them will stay. When we combine with liquid chromatography, it would be better. In this paper, the only component that can be analysed is presented.

This work has also some homework to solve, the unseparated compounds around 49 to 50 minutes retention time that showed up in each time during chromatographic separation (Figure 1). The unresolved spectrum might due to similar retention times of some similar compounds, but have more attachment to the silica surface. This might come as heavy and long chained and nonpolar hydrocarbon, and those would not be separated easily by gas chromatography.

4. Conclusion

The different chemical components in robusta, Arabica, and peaberry beans coffee were presented. Separation using extraction methods followed by chromatography and mass spectrometry were demonstrated. The chemicals contribute to taste as well as the aroma of the hot brewed coffee were inherently different in the types of coffee beans. The extraction, as well as separation methods to describe coffee chemical components, can still be enhanced using more modern instrumentation.

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